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Analytical Quality by Design Approach in RP-HPLC Method Development for the Quantification of Mirabegron and Solifenacin Succinate in Pharmaceutical Formulation

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Abstract: For concurrent measurement of mirabegron and solifenacin succinate in pharmaceutical formulations, a simple, sensitive, precise, accurate, and robust high-performance liquid chromatography approach has been established. For multivariate optimization of the RP-HPLC method's experimental conditions, the design of experiments (DoE) was used. A risk analysis was done to determine the crucial technical parameters. Three independent factors, % acetonitrile, buffer strength, and mobile phase pH, were used to design mathematical models. The response surface approach and the impacts of these independent elements were thoroughly examined using central composite design (CCD). Contour diagram predictions and optimizations included acetonitrile (55 % v/v) and phosphate buffer (15 mM) of pH 6 at a 1 ml/min flow rate and analytical wavelength of 272 nm. Under these optimal conditions, baseline separation of both medications was accomplished with superior resolution and a run duration of less than 6 minutes. According to ICH criteria, the improved assay conditions were validated. Thus, it was evident from the findings that the quality-by-design approach could be effectively used to improve the RP-HPLC technique for the simultaneous measurement of mirabegron and solifenacin succinate.

Keywords: mirabegron; solifenacinsuccinate; quality by design; RP-HPLC.

1. Introduction

A new combination of mirabegron (MB) and solifenacin succinate (SFS) for treating overactive bladder has recently received approval. The combination of the beta-3 adrenoreceptor agonist mirabegron and the counter muscarinic specialist Solifenacin succinate may further develop adequacy in treating overactive bladder OAB) while decreasing the anti-muscarinic side effects. Mirabegron is an agonist of β-3 adrenergic receptor. The chemical name is (2-amino-1,3-thiazol-4-yl)-N-[4-(2-[[(2R)-2-hydroxy-2-phenylethyl]amino]ethyl]phenyl]acetamide (Figure1) having molecular formula $C_{21}H_{24}N_4O_2S$ and molecular weight 396.5 g/mol. Its pKa1 is (4.2) while its pKa2 is (8.0). Mirabegron is a crystalline substance with a white appearance. The compound exhibits a high degree of insolubility in aqueous solutions. The compound exhibits solubility in methanol and dimethyl sulfoxide. The melting point of the substance is within the range of 138-140°C. It is classified

as Class 3 in the biopharmaceutical classification system [1]. Solifenacin Succinate is an anti-muscarinic selective M3 / anti-cholinergic category agent. The chemical name is [(3R)-1-azabicyclo[2.2.2]octan-3-yl](1S)-1-phenyl-3,4-dihydro-1H-isoquinoline-2-carboxylate; butanedioic acid (Figure 1) having molecular formula $C_{23}H_{26}N_2O_2;C_4H_6O_4$ and molecular weight 480.6 g/mole. It has a pKa (8.0). Solifenacin Succinate (SFS) is available as white crystals or powder. It dissolves easily in water. It is soluble in methanol as well. The melting point varies from 134 to 136°C. It falls within the biopharmaceutical classification system's Class 1 category [2]. RP-HPLC, UPLC, HPTLC, and spectrophotometric methods for the estimation of MB in combination with other drugs are reported [3-10]. The literature survey revealed the report of LC-MS/MS, HPLC, LCMS, and spectrophotometric methods for estimating SFS [11-26]. For simultaneous quantification of MB and SFS, HPTLC [27] and HPLC [28,29] methods were reported. According to recent FDA guidelines, these methodologies did not adequately define the design space. It is advised to employ "Quality by Design" (QbD) or "Design of Experiments" (DoE) to establish robustness during the validation of analytical methods through statistical quality control monitoring and the investigation of factors that have a negative impact on the quality in pharmaceutical analysis. The traditional process of method development involves trial and error and modifying one aspect at a time. Due to many considerations, such as the restricted availability of the chromatographic column, solvents, chemicals, and important physicochemical features of the analyte, this technique frequently finds challenges in setting robust chromatographic conditions. The FDA has recently authorized a few new drug applications (NDA) that have adapted the QbD methodology to analytical techniques, including HPLC and UV Spectrophotometry, allowing regulatory flexibility for movement within the specified method operational design region (MODR). Since the FDA implemented QbD, the pharmaceutical product development process has become more robust. A contemporary QbD-based treatment of the robustness of the HPLC method necessitates the evaluation of all variables that have the greatest impact on the method's outcomes. It is impractical, challenging, and more expensive to experimentally verify multiple factors at once. A detailed understanding of the response of the system quality to system parameters, which eventually results in the formation of the design space for the method, is crucial to overcoming the difficulty and reducing the experimental burden. Consequently, we developed a simple, rapid, precise, and robust RP HPLC method for the analysis of mirabegron and solifenacin succinate, aided by the design of experiments and a central composite design (CCD) for evaluating the robustness of the developed method, followed by the graphical interpretation of data by response surface methodology (RSM).

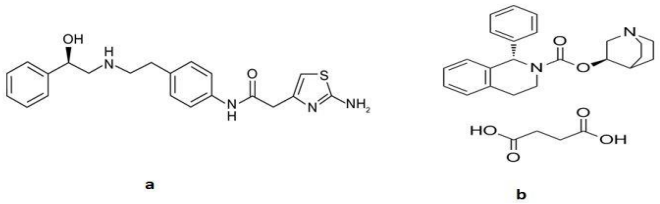


Figure 1. Structure of **(a)** mirabegron; **(b)** solifenacin succinate.

2. Materials and Methods

2.1. Chemicals and reagents.

The pharma industry developed both drug standards. Methanol, water, and acetonitrile (LC grade) were purchased from Merck India Ltd, Mumbai. Potassium dihydrogen phosphate

and orthophosphoric acid were from SD Fine Chemicals Mumbai. Miragron S 50 (Mirabegron 50 mg, Solifenacinsuccinate 5 mg, Steris Healthcare Private Limited) was obtained from a local pharmacy.

2.2. HPLC instrumental condition.

HPLC analysis was performed using a reversed-phase column-based high-performance liquid chromatography technique (CYBERLAB, USA). The apparatus was composed of an LC-P100 binary pump and a variable wavelength programmable LC-UV 100 detector. A Rheodyne injector with a 20 µL loop was used, and LC Solutions software version 4.0 was used to record and analyze the results. Digital Microbalance (SHIMADZU AUX 220) was used to weigh materials. The drug and sample solution was sonicated using a Citizen ultra sonicator. Before being used, the solvents were filtered through a 0.22 µm membrane filter and degassed in an ultrasonic bath. The mobile phase was used as diluent.

2.3. Software.

The desirability function, data analysis, and experimental design (CCD) were calculated using Design-Expert version 12.

2.4. Preparation of buffer solutions.

0.11 grams of potassium dihydrogen orthophosphate was weighed into a 1000 ml beaker, and 1.41 g of monobasic potassium phosphate was added and diluted to 1000 ml with HPLC water.

2.5. Preparation of standard solutions.

A sample containing 25 mg of each medication is weighed and transferred to a volumetric flask measuring 25 ml. After adding 15 ml of acetonitrile, the solution is sonicated for 15 minutes. To create a stock solution containing 1000 µg/ml, the volume is brought up to the required level with acetonitrile. To obtain working standard solutions of 100 µg/ml, 2.5 ml are taken from the standard stock solutions and transferred to 25 ml volumetric flasks. The volume is then brought up to the required level with the mobile phase.

2.6. Preparation of pharmaceutical samples.

Miragron S 50 containing 50 mg Mirabegron and 5 mg solifenacin succinate was weighed, the average weight was calculated, and powder equivalent to 5 mg and 1 mg of MB and SFS was weighed accurately and transferred into a 10 ml clean, dry volumetric flask. The flask's contents were dissolved in a diluent, subjected to a 30-minute sonication, built up to the required volume, and labeled as a sample stock solution. PVDF 0.45 µm filters were used to filter the sample stock solution. Transferring 1 ml of the filtered sample stock solution to a 10 ml volumetric flask, the volume was then brought up to 10 ml with diluent.

2.7. Software-aided method optimization.

The significant chromatographic factors in the current investigation were chosen based on preliminary studies and prior knowledge from the literature. Because of its efficiency in terms of the number of runs required, CCD may be a good choice among screening designs in assessing a few components (three or fewer) for robustness. For method development, several

aspects were considered, including the amount of organic solvents in the mobile phase and the pH of the buffer. As a result, CCD was used to assess the effects of three independent chromatography parameters on three defined critical response variables. The design included 20 experimental runs and aided in factor screening by assessing each component's main effect to obtain the study's outcomes. Two levels and three factors were included according to a 32-factorial design. The two levels were low (-1) and high (+1), whereas factors were (X_1) proportion of acetonitrile in the mobile phase (45 % and 65 %), (X_2) buffer strength (10 and 20 mM), and (X_3) pH of buffer (5.5 and 6.5). The resolution factor of MB (Y_1) and theoretical plates of SFS (Y_2) were used as responses in the experimental design and were shown in Table 1. The data obtained were incorporated into Design-Expert version 12. To analyze the behavior of the response around optimal values of the factors and to get the highest system performance, the response surface quadratic approach was found to be a viable method. Analysis of variance (ANOVA) was used to investigate the model's significance. Conditions were chosen from this optimized technique and verified for method performance, including accuracy, precision (less than 2% RSD), and robustness as a targeted response. Table 2 describes the conditions and observed responses for twenty experiments.

Table 1. An experimental plan of CCD shows factors with levels.

Factor	**Code**	**Range levels**	
		Low(-1)	**High(+1)**
% Acetonitrile composition in the mobile phase	X_1	45	65
Buffer strength	X_2	10	20
pH of Buffer	X_3	5.5	6.5
Responses			
Resolution factor of MB	Y_1	-	-
Theoretical plates of SFS	Y_2		-

Table 2. Coded values for factor level and observed responses in CCD for 20 analytical trials.

Experiment (Run)	**Type**	**X_1**	**X_2**	**X_3**	**Y_1**	**Y_2**
1	Center	45	20	5.5	3.785	4835
2	Axial	45	10	5.5	2.412	3876
3	Axial	65	20	5.5	3.893	5694
4	Axial	55	15	5.1591	4.562	4934
5	Axial	65	10	6.5	3.592	4123
6	Center	45	10	6.5	2.895	3345
7	Factorial	55	15	6.8409	3.902	5231
8	Factorial	55	23.409	6	4.892	5123
9	Center	55	15	6	3.463	4867
10	Factorial	55	6.59104	6	3.671	2672
11	Factorial	45	20	6.5	3.932	3893
12	Center	65	20	6.5	4.012	5123
13	Factorial	38.1821	15	6	2.123	4623
14	Factorial	71.8179	15	6	4.452	6723
15	Axial	55	15	6	3.123	4867
16	Axial	65	10	5.5	3.459	4980
17	Factorial	55	15	6	3.463	4867
18	Center	55	15	6	3.463	4867
19	Center	55	15	6	3.463	4867
20	Factorial	55	15	6	3.463	4867

2.8. Method validation.

The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) (2005) Q2R(1) guidelines [30] for system suitability,

linearity, limit of detection, limit of quantitation, precision, accuracy, specificity, and robustness.

2.8.1. System suitability test.

By injecting six replicates of standard solutions containing 50 µg/ml of MB and 10 µg/ml of SFS before to the sample analysis, system suitability characteristics such as number of theoretical plates, resolution, and tailing factor were assessed. The percent relative standard deviation in each situation should be less than 2.0%. In each chromatogram, the acceptability criteria for standards in system suitability were established.

2.8.2. Linearity.

The new method's linearity was established at six levels across the concentration ranges of 25–125 µg/ml for MB and 5–25 µg/ml for SFS. Each linearity solution was administered in triplicate using the appropriate sample concentrations. Using linear regression analysis, the calibration curve was created by graphing the peak area against the concentration.

2.8.3. accuracy and precision.

Accuracy was tested by adding a known amount of standard to each drug's tablet solution in triplicate at 50, 100, and 150% levels, and samples were analyzed using the optimized procedure. The percentage recovery for both medicines was then computed. Acceptance was set at 100% ± 2% recovery of the target concentrations. The optimal method's precision was determined by examining the intermediate precision and repeatability. Intermediate precision is expressed in laboratory variances such as different days, analyzers, equipment, etc. Inter-assay precision is another name for intermediate precision. Repeatability expresses precision across a short time interval under the same operating conditions. The method's precision was analyzed by six homogeneous MB and SFS samples.

2.8.4. Limit of detection (LOD) and limit of quantitation (LOQ).

The standard deviation method evaluated LOD and LOQ of MB and SFS. LOD was defined as 3.3 σ/S and LOQ as 10 σ/S based on the standard deviation of the response (σ) and the calibration curve (S) slope.

2.8.5. robustness.

The method's robustness refers to its capacity to remain unaffected by tiny and deliberate modifications in method parameters. The robustness of the optimized method was evaluated by injecting the system suitability solution with minute deliberate variations in the chromatographies parameters proportion of solvent in the mobile phase, flow rate of the mobile phase, and analytical wavelength. It was calculated as a percentage of relative standard deviation. Materials and methods should be described with sufficient details to allow others to replicate and build on published results. Please note that the publication of your manuscript implies that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose any restrictions on the availability of materials or information at the submission stage. New methods and protocols should be described in detail, while well-established methods can be briefly described and appropriately cited.

3. Results and Discussion

3.1. Preliminary studies and factor selection.

A preliminary investigation was conducted in order to find a simple, robust, and cost-effective RP-HPLC method for estimating MB and SFS in mixtures. The relevant chromatographic factors were chosen based on preliminary investigations and prior publications from the literature. Such research to choose factor levels for screening and optimization studies found that mobile phase conditions required to be improved so that both MB and SFS could be separated in a short run time. The mobile phase composition of phosphate buffer pH, acetonitrile volume, and buffer concentration turned out to be more appropriate for the simultaneous estimation of both medications, resulting in a significant shift in resolution. As a result, it is regarded as one of the key parameters in method development.

3.2. QbD-assisted method development.

This work used the CCD design to optimize the analytical procedure. A thorough and effective experimental plan of the RP-HPLC method is presented based on carefully examining three vital components(volume of acetonitrile, buffer strength, and pH of buffer). The CCD design was employed in the present analytical method optimization study.

To verify the robustness of the RP-HPLC method, a multivariate approach DoE with CCD was used to examine the simultaneous variations of the factors on the responses taken into account, such as the resolution factor of the MB (Y_1) and theoretical plates of SFS (Y_2). The experimental levels with factors are shown in Table 1. Based on the effects of three factors on responses and the examination of these data (Table 2), it was possible to develop mathematical models that attempted to determine the relationship between the factors and the examined responses. The response surface quadratic model was shown to be the best-fitting model for CCD. ANOVA was also used to validate the model using Design Expert software. The predicted R- R-squared values of the resolution factor of MB (Y_1) and theoretical plates of SFS (Y_2) were in reasonable agreement with adjusted R- squared values, i.e., the difference is less than 0.2. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable, and the responses obtained for the Y_1 and Y_2 were 7.79 and 12.52, respectively, which indicates adequate precision. This quadratic model for Y_2 and linear model for Y_1 can be used to navigate the design space. Model F-value of responses for a resolution of MB (Y_1) and theoretical plates of SFS (Y_2) were 6.48 and 8.04, which implies the model is significant. Hence, the values of significant responses showed p value < 0.05, suggesting that the model terms are significant. The low standard deviation and high adjusted R-square value indicate a good relationship between experimental data and those of fitted models. The data is represented in Table 3. The equations in terms of coded factors can be used to make predictions about the response for given levels of each factor. This equation is useful for identifying the relative impact of the factors by comparing the factor coefficient. Final equations for Y_1, and Y_2 are:

$$MB(Y_1) = +3.60 + 0.4283X_1 + 0.3894X_2 - 0.0167X_3$$

$$SFS\ (Y_2) = +5.4879 + 549.38X_1 + 537.68X_2 - 175.85X_3 + 25.87X_1X_2 + 5.62X_1X_3 - 15.63X_2X_3 + 200.18X_1^2 - 427.5X_2^2 - 8.59X_3^2$$

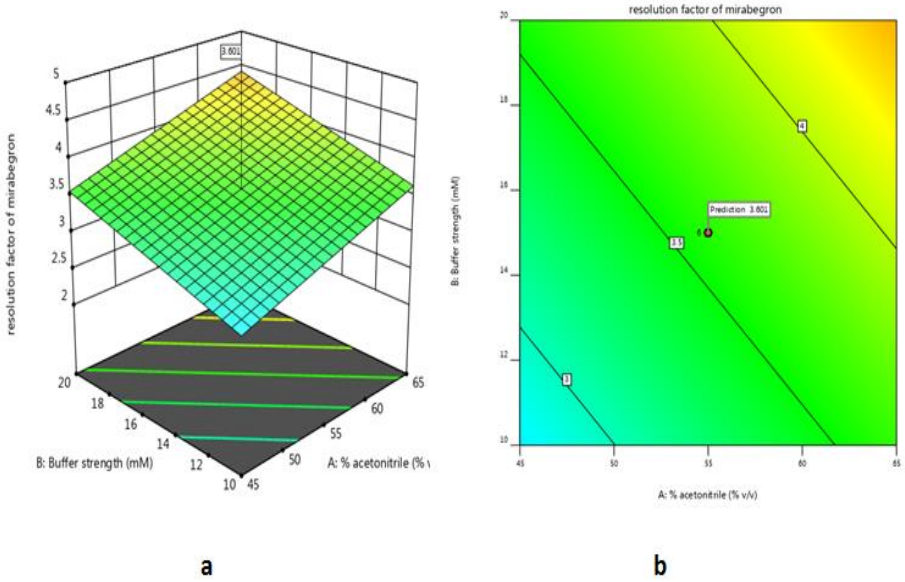
Table 3. ANOVA regression analysis for models and responses.

Response	Mean	SD	%CV	Press value	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	F	P
Resolution of MB (Y_1)	3.60	0.4789	13.3	6.47	0.9537	0.9221	0.9121	7.79	6.66	0.004
Theoretical plates of SFS (Y_2)	4718	407.01	8.63	12876	0.9947	0.9199	0.9229	12.52	8.04	0.0016

a=Standard deviation; b=Coefficient of variations; c=Coefficient of Regression; d=Fischer's ratio

As per the values of the coefficient from the above equations and their signs, it is clear that factors such as acetonitrile volume (X_1) and buffer strength (X_2) have a positive effect on the resolution of MB (Y_1) and theoretical plates of SFS (Y_2). The buffer pH (X_3) negatively affected the retention time of MB and SFS, Y_1 and Y_2. The pH of the buffer (X_3) had a negative effect on the resolution of MB (Y_1) and theoretical plates of SFS (Y_2). Interactions of X_1 and X_2 had a positive effect on Y_2; X_2 and X_3 had a negative effect on Y_2; X_1 and X_3 had a positive effect on Y_2. The squares of factors, X_1^2 had positive effects on Y_2; X_2^2 and X_3^2 had negative effects on Y_2.

Response surface and contour plots were analyzed to visualize the effect of the factors and their interactions on the response. The contour plots showed curvature, displaying a nonlinear effect of factors on responses. Figures 2 and 3 showed 2D (A) and 3D (B) contour plots displaying the effect of acetonitrile volume (X_1) and buffer strength (X_2) on the resolution of MB (Y_1) and theoretical plates of SFS (Y_2). A linear increasing trend was observed for the acetonitrile volume (X_1) and buffer strength (X_2) with respect to the resolution of MB Y_1, and a curvilinear increasing trend was observed for the acetonitrile volume (X_1) and buffer strength (X_2) with respect to theoretical plates of SFS (Y_2). An increasing curvature trend was observed for both X_1 and X_2, which showed higher resolution at higher levels. Therefore, optimized levels of X_1 and X_2 were recommended to achieve resolution.



a b

Figure 2. (a) 3D plot; **(b)** 2D plot for the study of effect factors on the resolution of MB (Y_1).

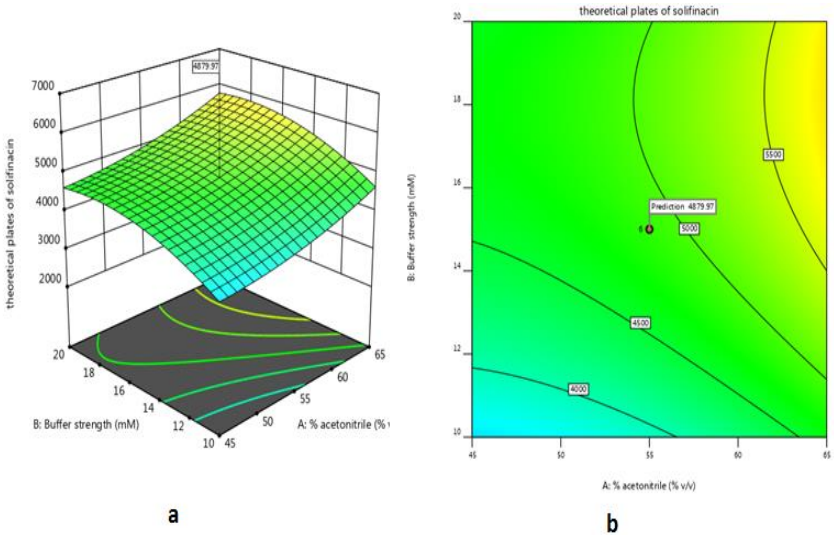


Figure 3. (a) 3D plot; **(b)** 2D for the study of effect factors on the theoretical plates of SFS (Y_2).

In order to find the ideal conditions based on the defined goals and boundaries for each response, a composite desirability was used. The desired goals were achieved within the established limits by the desirability function "R," which is equal to unity, and the whole experimental area was explored for the compositions, where the constraints set were met to the maximum, i.e., unity. The box design for the model is shown in Figure 4. The optimum values of chromatographic conditions of RP-HPLC were selected as % acetonitrile (X_1) of 55 % v/v, buffer strength (X_2) of 15 mM, and buffer pH 6. which resulted in a resolution of MB (Y_1) 3.42±0.0162 and theoretical plates of SFS (Y_2) 4712 ± 1.213, respectively, as shown in Figure 5.

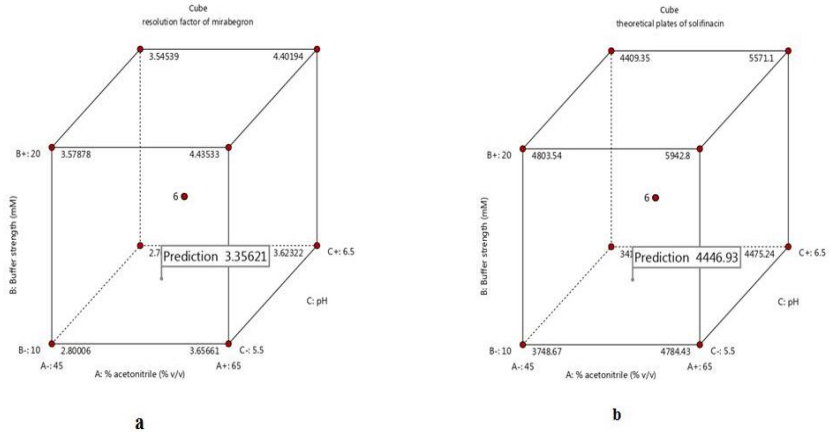


Figure 4. Prediction plot for **(a)** resolution of MB (Y_1); **(b)** theoretical plates of SFS (Y_2).

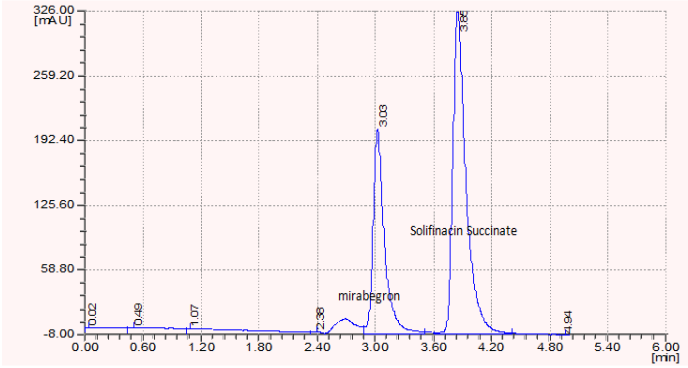


Figure 5. Chromatogram of mirabegron and solifenacin succinate from the optimized chromatographic conditions.

3.3. Method validation.

System suitability evaluations, in accordance with the ICH, are a crucial component of liquid chromatographic methods. The column efficiency, as evaluated by the number of theoretical plates for both medicines, was greater than 2000, the resolution was 3.42, and the tailing was less than 2. The percent relative standard deviation for six replicate injections was found to be 0.87 in the given concentration of 50 µg/ml for MB and 1.09 in the given concentration of 10 µg/ml for SFS, respectively. As % RSD was found to be less than 2%, it has shown good injection repeatability. Linearity of the developed method was confirmed by plotting the linearity curve over concentrations ranging from 25-125 µg/ml for MB and 5-25 µg/ml for SFS, with a correlation coefficient (r^2=0.999) for both the drugs, shown in Table 4. The representative chromatogram is shown in Figure 6. The obtained correlation coefficient (r^2=0.999) demonstrates an excellent correlation between peak area and concentration. For the recovery study, different concentrations of samples (50, 100, and 150%) of standard concentrations for both drugs were prepared and showed recovery of 99.3-101.43 % and 99.02-99.89% for MB and SFS, respectively. Data is shown in Table 3, indicating that the developed method has a high level of accuracy with % RSD 0.87 and 0.73 for MB and SFS, respectively. Intermediate precision and repeatability were carried out, and the resultant data are given in Table 3. The precision values for both drugs were less than 2 %., indicating that the method was repeatable and precise. The LOD and LOQ were found to be 0.26 and 0.78 µg/ml, respectively, for MB, and 0.18 and 0.54 µg/ml, respectively, for SFS. The robustness of the RP-HPLC method to small variations in the optimum experimental modifications revealed its insensitivity to such minor alterations. The mobile phase composition and buffer pH significantly impacted MB and SFS's resolution and retention time.

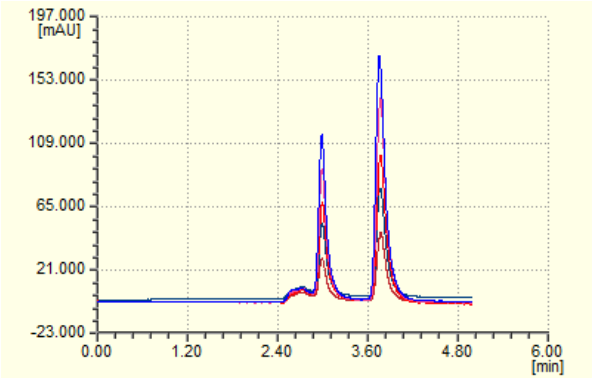


Figure 6. Linearity chromatogram.

Table 4. Results for validation studies.

Parameters	**MB**	**SFS**
Linearity	25- 125 µg/mL	5-25µg/mL
Sensitivity		
LOD	0.26 µg/mL	0.18 µg/mL
LOQ	0.78 µg/mL	0.54 µg/mL
Precision[a]		
Repeatability(% RSD)	0.412-1.013	0.219-0.987
Intermediate precision (% RSD)	1.021-1.459	0.877-0.912
Accuracy[b]		
Recovery studies	99.80-100.38	99.62-101.50
Robustness		
Change in flow rate(% RSD)	1.12	0.88
Change in mobile phase composition (% RSD)	0.89	0.72
Change in wavelength(% RSD)	1.11	1.01

4. Conclusions

For the first time, the current study involves the systematic QbD-based creation of a simple, rapid, precise, and cost-effective RP-HPLC method for simultaneous quantification of MB and SFS. The experimental design describes scouting important components, such as acetonitrile volume, buffer strength, and pH. The modeling software aided in a better understanding of the elements impacting the optimization of the procedure and separation of MB and SFS. CCD was used to optimize the resolution as a response between MB and SFS in a reasonably short time (6 minutes). The improved model's acetonitrile and phosphate buffer (15 mM; pH 6.0) ratio of 55:45 v/v reveals its suitability for estimating MB and SFS. The validation study confirmed the selection of the optimum conditions by proving that the method was selective, specific, accurate, linear, accurate, and robust. As a result, using the response surface technique provides superior insight for method development and robustness testing. This created technique meets the design space idea and is eligible for regulatory submission under regulatory flexibility.

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Conflicts of Interest

The authors declare no conflict of interest.

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